Purpose: The infrapatellar fat pad (IPFP) is commonly resected during knee joint arthroplasty. This longitudinal study examined the associations between the maximum cross-sectional area (CSA) of the IPFP and knee cartilage volume and pain in adults without knee osteoarthritis (OA).
Methods: 297 adults without baseline knee pain or a diagnosis of knee OA had MRI performed at baseline and follow-up (n = 271). IPFP maximal CSA and tibial cartilage volume were measured from MRI. Body composition was performed at baseline using bio-impedance. Knee pain was assessed at follow-up using the Western Ontario and McMaster University Osteoarthritis Index (WOMAC).
Results: A larger IPFP at baseline was associated with reduced knee pain at follow-up (OR 0.5, 95% CI: 0.3 to 0.9, p = 0.02) and lateral tibial cartilage volume loss (β: -0.9% (95% CI: -1.6, -0.1%) per annum, p = 0.03). The maximal CSA of the IPFP was predominantly located in the lateral (54.2%), rather than the medial tibiofemoral compartment (1.7%). Male gender (OR 12.0, 95% CI: 6.5 to 22.0, p < 0.001) and fat free mass (OR 1.15, 95% CI 1.04 to 1.28, p = 0.007) were both associated with a large IPFP.
Conclusions: A larger IPFP predicts reduced lateral tibial cartilage volume loss and knee pain and mechanistically might function as a local shock-absorber. The lack of association between measures of adiposity and the size of the IPFP might suggest that the IPFP size is not simply a marker of systemic obesity.
OA: Cartilage and Bone

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HIGH SYSTEMIC LDL CHOLESTEROL LEVELS DURING EXPERIMENTAL OSTEOARTHRITIS LEAD TO INCREASED SYNOVIAL ACTIVATION AND ECTOPIC BONE FORMATION AT END-STAGE OSTEOARTHRITIS, WHILE EXCESSIVE LEVELS ACCELERATE DEVELOPMENT OF JOINT PATHOLOGY ALREADY AT EARLY-STAGE OF OSTEOARTHRITIS

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Purpose: A relation between osteoarthritis (OA) and the metabolic syndrome has long been established. One of the characteristics of the metabolic syndrome is increased cholesterol levels. In a recent study, we showed that LDL accumulation by LDL receptor deficient mice resulted in increased ectopic bone formation during experimental osteoarthritis.
In the present study we investigate OA pathology in ApoE deficient ($ApoE^{-/-}$) mice with and without a cholesterol-rich diet, which is a model for extremely high systemic LDL cholesterol levels.
Methods: Wild type (WT) and $ApoE^{-/-}$ mice received a normal or cholesterol-rich diet for 54 days. At day 18, experimental OA was induced by intra-articular injection of collagenase and animals were sacrificed at day 28 and 54. Gene expression in synovium was measured by RT-PCR and joint pathology was investigated by histology. LDL levels were measured in serum and synovial wash-outs.
Results: $ApoE^{-/-}$ mice on a normal diet showed markedly higher LDL levels than WT mice (8.90 mmol/L and 0.40 mmol/L, respectively; p<0.001). While no differences between the two groups were found at the early time point (day 28), end point OA (day 54) in $ApoE^{-/-}$ mice showed a strong increase of ectopic bone formation, mainly at the medial collateral ligament (fold increase 5.4; p<0.001) compared to WT mice. No significant differences in cartilage damage were found between the two groups; a slight increase in synovial thickening, however, was found in $ApoE^{-/-}$mice (arbitrary score 1.9 versus 1.1 in WT mice; p<0.05). Furthermore, synovial gene expression of both S100A8 and S100A9 (fold increase 1.8 and 1.4, respectively; p<0.05) and S100A8/S100A9 protein levels of synovial wash-outs were increased in ApoE-/- mice (fold increase 5.8; p<0.05), suggesting an activated status of synovial lining cells.
In addition, we investigated whether a cholesterol-rich diet could increase joint pathology after induction of OA. The diet increased LDL levels even more in $ApoE^{-/-}$ mice (fold increase 2.1, compared to $ApoE^{-/-}$ mice on a normal diet; p<0.001). In both $ApoE^{-/-}$ and WT mice on a cholesterol-rich diet, excessive bone formation was found in the medial collateral ligament at day 54, however, no significant difference was found between the two groups. Interestingly, at the early time point (day 28; 10 days after OA induction), histological differences between the two groups were observed. Synovial thickening was four times increased (p<0.001) in $ApoE^{-/-}$mice on a cholesterol-rich diet and also ectopic cartilage formation in the medial collateral ligament was strongly increased (fold increase 2.7; p<0.01) compared to WT mice on a cholesterol-rich diet.
Conclusions: LDL cholesterol accumulation by ApoE deficiency or a cholesterol-rich diet results in increased synovial activation and ectopic bone formation in experimental OA. Excessive LDL levels induced by a combination of ApoE deficiency and a cholesterol-rich diet strongly elevated synovial activation and ectopic bone formation at an early stage of the disease without affecting cartilage destruction.

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EXPERIMENTAL EVALUATION OF DISCOIDIN DOMAIN RECEPTOR 2 AS AN IDEAL TARGET FOR DEVELOPMENT OF DISEASE-MODIFYING OSTEOARTHRITIS DRUGS

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Purpose: The goal of this study is to evaluate if the complete removal of Ddr2 from the knee joint of mouse adult articular cartilage can delay progression of osteoarthritis prior to or after initiation of articular cartilage degeneration. MMP-13, which has the ability to degrade both aggrecans and type II collagen, would be an ideal target for disease-modifying osteoarthritis drugs. However, the broad biological effects of MMP-13 restrict its application as a target enzyme of inhibitor drugs in the treatment of OA. Recent studies demonstrate that a specific amino acid sequence, from amino acid 594 to 605, on type II collagen is preferentially recognized by DDR2. The expression and subsequent activation of DDR2 were increased in human OA tissues and mouse models of OA, and this was co-localized with elevated expression of MMP-13 in degenerative articular cartilages. Conversely, reduced expression of Ddr2 in the heterozygous Ddr2 knockout condition attenuated progression of articular cartilage degeneration in mouse models of OA. Typically, DDR2 is kept inactivated by the presence of the pericellular matrix, which separates chondrocytes from type II collage in healthy articular cartilage. Once enzymes, such as a serine proteinase, high temperature requirement A1, that are capable of degrading the pericellular molecules expose chondrocytes to type II collagen, DDR2 is activated and then induces expression of MMP-13 leading the degradation of type II collage and proteoglycans resulting in joint destruction and OA.
Methods: 1) By use of conditional knock out techniques with aggrecan-CreERt2 mice and floxed Ddr2 mice, Ddr2 was removed from articular cartilage of knee joints in mice at 8 weeks of age via intraperitoneal injection Tamoxifen injection (2mg/10g body weight) for 5 consecutive days (Group A). Mice were subjected to destabilization of the medial meniscus (DMM) or sham surgery at 10 weeks of age. 2) An additional experimental group was subjected to DMM or sham surgery at 10 weeks of age and then Ddr2 was removed by intraperitoneal injection Tamoxifen injection 8 weeks later (Group B). 3) Knee joints from Groups A and B mice and their corresponding controls (n=7) were then collected for morphological analysis. Knee joints from mice in Group A were harvested at 8 weeks (n=7) or 16 weeks (n=9) post-surgery and those from Group B (n=5) at 16 weeks post-surgery. 4) Histology was performed. 5) ORASI Modified Mankin Score was used to evaluate articular cartilage degeneration. 6) Statistically significant differences were determined via T-test.
Results: 1) The average modified score for Group A 8 week control was 1.64 (score range 1.5-2) whereas the average modified score with Ddr2 removed was 0.64 (score range 0.5-1) [P<0.05]. 2) The average modified score for Group A 16 week control was 4.67 (score range 4-5) and the average modified score with Ddr2 removed was 1.27 (score range 0.5-3) [P<0.05]. 3) The average modified score for Group B was 1.1 (score range 0.5-2).
Conclusions: Conditional removal of Ddr2 in articular cartilage attenuated articular cartilage degeneration in mature knee joints of mouse models of OA. Therefore, inhibiting activity of DDR2, may be considered in treatment of OA in mature joints in humans.

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ASSOCIATION BETWEEN CARTILAGE DEGENERATION AND SUBCHONDRAL BONE METABOLISM IN PATIENTS WITH KNEE OSTEOARTHRITIS

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Purpose: The purpose of this retrospective cross-sectional study was to investigate the association between cartilage lesions assessed with 3T MRI and remodeling of the subchondral bone detected by 99mTc-DPD-SPECT/CT.
Methods: 99mTc-DPD-SPECT/CT and MRI of 27 knees of 25 patients with chronic knee pain and risk factors for osteoarthritis were evaluated independently by one nuclear physician and one radiologist. Six regions (patella, trochlea, medial/lateral tibia, medial/lateral femur) were visually assessed according to structural joint lesions graded with a modified Whole Organ MR imaging score (WORMS) and according to subchondral 99mTc-DPD-SPECT uptake visually graded with 0=normal uptake, 1=moderately elevated uptake, 2=severely elevated uptake. Relationships between lesion scores and uptake were quantified using two methods: 1. For the global assessment per joint, we used a linear regression and Spearman correlations and 2. For the assessment of each of 162 regions, general estimating equations was used to control for multiple measurements per subject.
Results: Elevated subchondral uptake was significantly associated with the grade ($p<0.0001$) and with the depth of cartilage lesions ($p<0.0001$). A similar association was observed between bone marrow edema pattern (BMEP) lesions and cartilage lesions.
Conclusions: Both, functional and structural changes of the subchondral bone in terms of scintigraphic osseous activity and the presence and degree of BMEP lesions were significantly associated with cartilage lesions in patients with osteoarthritis of the knee. This association was pronounced with full thickness lesions indicating a possible protective effect of the cartilage layer for the subjacent bone.

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REDUCED EGF RECEPTOR SIGNALLING AGGRAVATES SIGNS OF POST-TRAUMATIC OSTEOARTHRITIS IN THE CALCIFIED TISSUES OF THE KNEE

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Purpose: Post-traumatic osteoarthritis (PTOA) develops in 40% of people following a significant knee injury. In contrast to spontaneous OA where diagnosis occurs with end stage disease when patients present with pain, the time between injury and end stage PTOA offers a critical opportunity to intervene in the disease process before debilitating signs or symptoms of OA are apparent.
Two key players in chondrocyte homeostasis are integrins and growth factor receptors. The collagen receptor integrin $\alpha1\beta1$ negatively regulates epidermal growth factor receptor (EGFR) signalling and its expression is increased in cartilage with spontaneous OA. Interestingly, integrin $\alpha1$-null mice develop spontaneous OA two months earlier than wildtype controls. Furthermore, Mig-6-null mice show increased EGFR signalling and accelerated spontaneous OA. The role of integrin $\alpha1\beta1$ in PTOA and the mechanism(s) by which this receptor exerts its influence upon OA are unknown. We hypothesized that integrin $\alpha1\beta1$ protects against PTOA by a mechanism that involves reduced EGFR activation and signalling.
Methods: All methods were approved by the University of Calgary Animal Care Committee. Surgery to destabilize the medial meniscus (DMM) or sham control surgery was performed on the left leg of 50 skeletally mature BALB/c male $\alpha1$-null and wildtype mice. Mice were sacrificed at 2, 4, 8 and 12 weeks post-surgery. An additional group of mice received the EGFR inhibitor erlotinib (50mg/kg/day by gavage) starting the day after surgery. At sacrifice, hind-limbs were isolated, skinned and fixed prior to microCT scanning. Bone mineral density and volume were calculated for the calcified portions of the menisci, fabella, medial collateral ligament, trabecular, and subchondral bone. Data were analyzed with ANOVA and Fisher LSD post hoc ($p<0.05$).
Results: There was no significant difference in mass between wildtype and integrin $\alpha1$-null mice (31.7 ± 0.4g vs 31.1 ± 0.3g, respectively). As expected, the anterior medial meniscus volume increased from 2 to 12 weeks only in the DMM surgery leg of both wildtype and $\alpha1$-null mice (Figure 1).
Mice in the erlotinib group had larger anterior medial meniscal volume at 12 weeks post-surgery compared to control, and this difference was greater in wildtype versus $\alpha1$-null mice (Figure 1). Among the 150 joints analyzed, 17 joints had a partially calcified medial collateral ligament (MCL). Interestingly, 7 (41%) of these joints belonged to the erlotinib

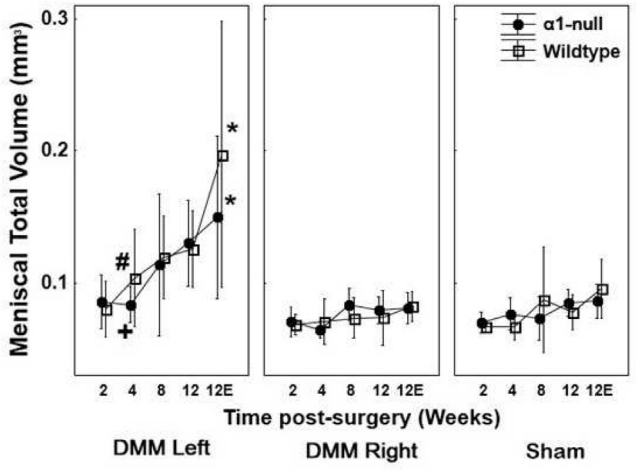

Figure 1. Total volume of anterior medial meniscus as a function of time post- surgery and genotype. 12E received erlotinib and 12 received vehicular control by daily gavage. Bars represent 95% confidence interval. Significantly ($p<0.05$) different from * = all other points, # = 2 and 12 week equivalent, + = 8 and 12 week equivalent.

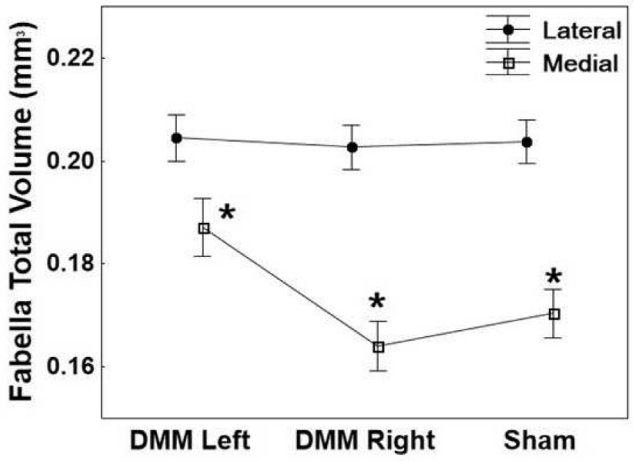

Figure 2. Total volume of fabella as a function of surgery type. Bars represent a 95% confidence interval. Significantly ($p<0.05$) different from * = all other points.

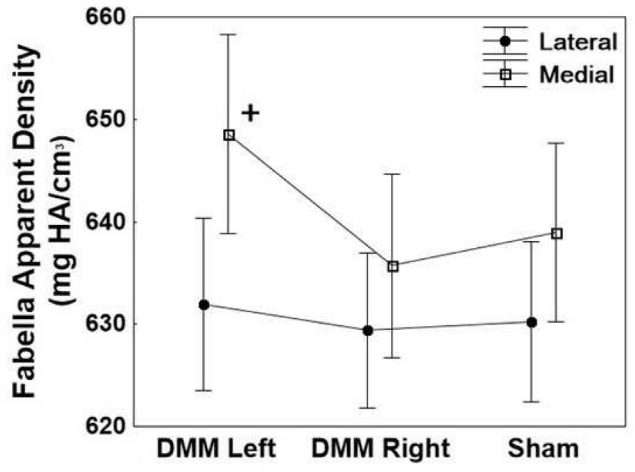

Figure 3. Apparent density of fabella as a function of surgery type. Bars represent a 95% confidence interval. Significantly ($p<0.05$) different from + = all other points except Sham equivalent.